Predicting the Substrate Scope of the Flavin-Dependent Halogenase BrvH

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The recently described flavin-dependent halogenase BrvH is able to catalyse both the bromination and chlorination of indole, but shows significantly higher bromination activity. BrvH was annotated as a tryptophan halogenase, but does not accept tryptophan as a substrate. Its native substrate remains unknown. A predictive model with the data available for BrvH was analysed. A training set of compounds tested *in vitro* was docked into the active site of a complete protein model based on the X-ray structure of BrvH. The atoms not resolved experimentally were modelled by using molecular mechanics force fields to obtain this protein model. Furthermore, docking poses for the substrates and known non-substrates have been calculated. Parameters like distance, partial charge and hybridization state were analysed to derive rules for predicting activity. With this model for activity of the BrvH, a virtual screening suggested several structures for potential substrates. Some of the compounds preselected in this way were tested *in vitro*, and several could be verified as convertible substrates. Based on information on halogenated natural products, a new dataset was created to specifically search for natural products as substrates/products, and virtual screening in this database yielded further hits.

Introduction

Halogenated metabolites are found in nature in many different organisms. They form an important part of the natural products family, and are most often found in marine invertebrates, algae, and bacteria. Bioactive molecules like pharmaceuticals or plant protectants frequently contain halogens, too. Some of the halogenated natural products are biologically active. For example, thienodolin is produced by *Streptomyces albogriseulus* and promotes plant growth.[1] Balhimycin from an *Actinomycete* species shows antibiotic activity against *Staphylococcus aureus*, *S. epidermis*, *S. haemolyticus*, and *Streptococcus* species.[2] Another halogenated antibiotic is produced by *Pseudomonas aeruginosa* and was named pyoluteorin.[3] Cryptophycins isolated from cyanobacteria show anti-tumour activity.[4] A *Salinospora* strain, producing the proteasome inhibitor salinosporamide A, is another marine source for highly bioactive compounds.[5] New halogenated metabolites are being identified constantly from different sources, like 6-bromo-8-oxo-conicamin A from the marine sponge *Oceanapia* sp. It shows micromolar activity against a human pancreatic cancer cell line.[6]

Chemical halogenation often requires harsh reaction conditions like Lewis acid, elevated temperature, and proceeds in organic solvents. The use of enzymes provides an elegant alternative since enzymes work in aqueous solution at room temperature. Flavin-dependent halogenases introduce halogen substituents in their substrates regioselectively and only require oxygen, a halide salt, and the reduced flavin $FADH_2$ as cofactor.[7] The most prominent members are the tryptophan halogenases, which catalyse the regioselective halogenation of tryptophan in different positions.[8] A flavin reductase is required for enzymatic cofactor regeneration with consumption of nicotinamide adenine dinucleotide (NADH). It was shown only recently that also NADH mimics can be employed instead of enzymatic cofactor regeneration in biocatalytic halogenation.[9] Furthermore, it is also possible to photochemically (455 nm) reduce bound FAD in the presence of EDTA.[10]

X-ray crystallographic structure analysis of PrnA helped to postulate a reaction mechanism for these enzymes.[11] $FADH_2$ reacts with oxygen to give a flavin hydroperoxide (FAD–OOH) which reacts with a halide nucleophile forming hypohalous acid (HOX). HOX then passes through a 10 Å tunnel to the substrate binding site. A highly conserved lysine residue (PrnA: K79) is important for the stabilization of HOX. It is still under debate whether HOX reacts with the lysine side chain giving an *N*-haloamine or whether there is an association by hydrogen bond formation.[11–13] In the substrate binding site, HOX or the haloamine react with the substrate in an electrophilic substitution reaction. A conserved glutamate residue (PrnA: E346) deprotonates the Wheland intermediate releasing the final halogenated product.[11,14] Recently, it was shown that substrate binding reduces the affinity for oxidized cofactor FAD to probably facilitate regeneration of $FADH_2$.[15]

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